

**WHAT IS CLAIMED IS:**

1. A method for detecting the presence or absence of *Bordetella pertussis* in  
5 a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an  
amplifying step and a hybridizing step, wherein said amplifying step comprises  
contacting said sample with a pair of IS481 primers to produce an IS481 amplification  
product if a *B. pertussis* IS481 nucleic acid molecule is present in said sample, wherein  
10 said hybridizing step comprises contacting said sample with a pair of IS481 probes,  
wherein the members of said pair of IS481 probes hybridize within no more than five  
nucleotides of each other, wherein a first IS481 probe of said pair of IS481 probes is  
labeled with a donor fluorescent moiety and a second IS481 probe of said pair of IS481  
probes is labeled with a corresponding acceptor fluorescent moiety; and

15 detecting the presence or absence of fluorescence resonance energy  
transfer (FRET) between said donor fluorescent moiety of said first IS481 probe and said  
corresponding acceptor fluorescent moiety of said second IS481 probe,

wherein the presence of FRET is indicative of the presence of *B. pertussis* in said  
biological sample, and wherein the absence of FRET is indicative of the absence of *B.*  
20 *pertussis* in said biological sample.

2. The method of claim 1, wherein said pair of IS481 primers comprises a  
first IS481 primer and a second IS481 primer, wherein said first IS481 primer comprises  
the sequence

25 5'-CCA GTT CCT CAA GGA CGC-3' (SEQ ID NO:1), and wherein said second  
IS481 primer comprises the sequence

5'-GAG TTC TGG TAG GTG TGA GCG TA-3' (SEQ ID NO:2).

3. The method of claim 1, wherein said first IS481 probe comprises the  
30 sequence

5'-CAC CGC TTT ACC CGA CCT TAC CGC CCA C- 3' (SEQ ID NO:3), and  
wherein said second IS481 probe comprises the sequence

5'-GAC CAA TGG CAA GGC CGA ACG CTT CAT C- 3' (SEQ ID NO:4).

5           4.       The method of claim 1, wherein the members of said pair of IS481 probes  
hybridize within no more than two nucleotides of each other.

5.       The method of claim 1, wherein the members of said pair of IS481 probes  
hybridize within no more than one nucleotide of each other.

10           6.       The method of claim 1, wherein said donor fluorescent moiety is  
fluorescein.

15           7.       The method of claim 1, wherein said acceptor fluorescent moiety is  
selected from the group consisting of LC<sup>TM</sup>-Red 640, LC<sup>TM</sup>-Red 705, Cy5, and Cy5.5.

20           8.       The method of claim 1, wherein said detecting step comprises exciting  
said biological sample at a wavelength absorbed by said donor fluorescent moiety and  
visualizing and/or measuring the wavelength emitted by said acceptor fluorescent moiety.

25           9.       The method of claim 1, wherein said detecting comprises quantitating said  
FRET.

          10.       The method of claim 1, wherein said detecting step is performed after each  
cycling step.

          11.       The method of claim 1, wherein said detecting step is performed in real-  
time.

30           12.       The method of claim 1, wherein the presence of said FRET within 40  
cycling steps is indicative of the presence of a *B. pertussis* infection in said individual.

13. The method of claim 1, wherein the presence of said FRET within 30 cycling steps is indicative of the presence of a *B. pertussis* infection in said individual.

5 14. The method of claim 1, wherein the presence of said FRET within 25 cycling steps is indicative of the presence of a *B. pertussis* infection in said individual.

15. The method of claim 1, further comprising preventing amplification of a contaminant nucleic acid.

10 16. The method of claim 15, wherein said preventing comprises performing said amplifying step in the presence of uracil.

15 17. The method of claim 16, wherein said preventing further comprises treating said biological sample with uracil-DNA glycosylase prior to a first amplifying step.

20 18. The method of claim 1, wherein said biological sample is selected from the group consisting of nasopharyngeal swabs, nasopharyngeal aspirates, and throat swabs.

19. The method of claim 1, wherein said cycling step is performed on a control sample.

25 20. The method of claim 19, wherein said control sample comprises said portion of said IS481 nucleic acid molecule.

30 21. The method of claim 1, wherein said cycling step uses a pair of control primers and a pair of control probes, wherein said control primers and said control probes are other than said IS481 primers and IS481 probes, respectively, wherein a control

amplification product is produced if control template is present in said sample, wherein said control probes hybridize to said control amplification product.

22. A method for detecting the presence or absence of *Bordetella*

*parapertussis* in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of IS1001 primer to produce an IS1001 amplification product if a *B. parapertussis* IS1001 nucleic acid molecule is present in said sample,

wherein said hybridizing step comprises contacting said biological sample with a pair of IS1001 probes, wherein the members of said pair of IS1001 probes hybridize within no more than five nucleotides of each other, wherein a first IS1001 probe of said pair of IS1001 probes is labeled with a donor fluorescent moiety and a second IS1001 probe of said pair of IS1001 probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first IS1001 probe and said corresponding acceptor fluorescent moiety of said second IS1001 probe,

wherein the presence of FRET is indicative of the presence of *B. parapertussis* in said biological sample, and wherein the absence of FRET is indicative of the absence of *B. parapertussis* in said biological sample.

23. The method of claim 22, wherein said pair of IS1001 primers comprises a first IS1001 primer and a second IS1001 primer, wherein said first IS1001 primer comprises the sequence

5'-GGC GAT ATC AAC GGG TGA-3' (SEQ ID NO:5), and wherein said second IS1001 primer comprises the sequence

5'-CAG GGC AAA CTC GTC CAT C-3' (SEQ ID NO:6).

24. The method of claim 22, wherein said first IS1001 probe comprises the sequence

5'-GTT CTT CGA ACT GGG TTG GCA TAC- 3' (SEQ ID NO:7), and wherein said second IS1001 probe comprises the sequence

5'-GTC AAG ACG CTG GAC AAG GCT C- 3' (SEQ ID NO:8).

25. A method for detecting the presence or absence of *Bordetella pertussis* and/or *B. parapertussis* in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises

contacting said sample with a pair of IS481 primers and a pair of IS1001 primers to produce an IS481 amplification product if a *B. pertussis* IS481 nucleic acid molecule is present in said sample and an IS1001 amplification product if a *B. parapertussis* IS1001 nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of IS481 probes and a pair of IS1001 probes, wherein the members of said pair of IS481 probes hybridize within no more than five nucleotides of each other and wherein the members of said pair of IS1001 probes hybridize within no more than five nucleotides of each other, wherein a first IS481 probe of said pair of IS481 probes is labeled with a donor fluorescent moiety and wherein a second IS481 probe of said pair of IS481 probes is labeled with a corresponding acceptor fluorescent moiety, wherein a first IS1001 probe of said pair of IS1001 probes is labeled with a donor fluorescent moiety and wherein a second IS1001 probe of said pair of IS1001 probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first IS481 probe and said corresponding acceptor fluorescent moiety of said second IS481 probe and/or between donor fluorescent moiety of said first IS1001 probe and said corresponding acceptor fluorescent moiety of said second IS1001 probe,

wherein the presence of FRET is indicative of the presence of *B. pertussis* and/or *B. parapertussis* in said biological sample, and wherein the absence of FRET is indicative of the absence of *B. pertussis* or *B. parapertussis* in said biological sample.

26. The method of claim 25, further comprising:

determining the melting temperature between one or both of said IS481 probes and said IS481 amplification product and between one or both of said IS1001 probes and said IS1001 amplification product, wherein said melting temperature(s) confirms said presence or absence of *B. pertussis* in said sample and said presence or  
5 absence of *B. paraptussis* in said sample.

27. The method of claim 26, wherein said melting temperature(s) distinguish between *B. pertussis* and *B. paraptussis* in said sample.

10 28. The method of claim 25, wherein said acceptor fluorescent moiety of said second IS481 probe and said acceptor fluorescent moiety of said second IS1001 probe are different.

29. An article of manufacture, comprising:  
15 a pair of IS481 primers;  
a pair of IS481 probes; and  
a first donor fluorescent moiety and a corresponding first acceptor fluorescent moiety.

20 30. The article of manufacture of claim 29, wherein said pair of IS481 primers comprise a first IS481 primer and a second IS481 primer, wherein said first IS481 primer comprises the sequence

5'-CCA GTT CCT CAA GGA CGC-3' (SEQ ID NO:1), and wherein said second IS481 primer comprises the sequence

25 5'-GAG TTC TGG TAG GTG TGA GCG TA-3' (SEQ ID NO:2).

31. The article of manufacture of claim 29, wherein said pair of IS481 probes comprises a first IS481 probe and a second IS481 probe, wherein said first IS481 probe comprises the sequence

30 5'-CAC CGC TTT ACC CGA CCT TAC CGC CCA C- 3' (SEQ ID NO:3), and wherein said second IS481 probe comprises the sequence

5'-GAC CAA TGG CAA GGC CGA ACG CTT CAT C- 3' (SEQ ID NO:4).

32. The article of manufacture of claim 29, wherein said first IS481 probe is labeled with said first donor fluorescent moiety and wherein said second IS481 probe is labeled with said corresponding first acceptor fluorescent moiety.

33. The article of manufacture of claim 29, further comprising:  
a pair of IS1001 primers;  
a pair of IS1001 probes; and  
a second donor fluorescent moiety and a corresponding second acceptor fluorescent moiety.

34. The article of manufacture of claim 33, wherein said pair of IS1001 primers comprise a first IS1001 primer and a second IS1001 primer, wherein said first IS1001 primer comprises the sequence  
5'-GGC GAT ATC AAC GGG TGA-3' (SEQ ID NO:5), and wherein said second IS1001 primer comprises the sequence  
5'-CAG GGC AAA CTC GTC CAT C-3' (SEQ ID NO:6).

35. The article of manufacture of claim 33, wherein said pair of IS1001 probes comprise a first IS1001 probe and a second IS1001 probe, wherein said first IS1001 probe comprises the sequence  
5'-GTT CTT CGA ACT GGG TTG GCA TAC- 3' (SEQ ID NO:7), and wherein said second IS1001 probe comprises the sequence  
5'-GTC AAG ACG CTG GAC AAG GCT C- 3' (SEQ ID NO:8).

36. The article of manufacture of claim 33, wherein said pair of IS1001 probes are labeled with said second donor fluorescent moiety and said corresponding second acceptor fluorescent moiety.

37. The article of manufacture of claim 33, further comprising a package insert having instructions thereon for using said pair of IS481 primers, said pair of IS481 probes, said pair of IS1001 primers and said pair of IS1001 probes to detect the presence or absence of *B. pertussis* and/or *B. paraptussis* in a biological sample.

38. The article of manufacture of claim 37, further comprising a package insert having instructions thereon for using said pair of IS481 probes and said pair of IS1001 probes to distinguish between *B. pertussis* and *B. paraptussis* in said biological sample.

39. An article of manufacture, comprising:  
a pair of IS1001 primers;  
a pair of IS1001 probes; and  
a donor fluorescent moiety and a corresponding acceptor fluorescent moiety.

40. A method for detecting the presence or absence of *B. pertussis* in a biological sample from an individual, said method comprising:  
performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of IS481 primers to produce an IS481 amplification product if a *B. pertussis* IS481 nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with an IS481 probe, wherein the IS481 probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of fluorescence resonance energy transfer (FRET) between said donor fluorescent moiety and said acceptor fluorescent moiety of said IS481 probe,

wherein the presence or absence of FRET is indicative of the presence or absence of *B. pertussis* in said sample.



41. The method of claim 40, wherein said amplification employs a polymerase enzyme having 5' to 3' exonuclease activity.

42. The method of claim 41, wherein said donor and acceptor fluorescent moieties are within no more than 5 nucleotides of each other on said probe.

43. The method of claim 42, wherein said acceptor fluorescent moiety is a quencher.

44. The method of claim 40, wherein said IS481 probe comprises a nucleic acid sequence that permits secondary structure formation, wherein said secondary structure formation results in spatial proximity between said donor and said acceptor fluorescent moiety.

45. The method of claim 44, wherein said acceptor fluorescent moiety is a quencher.

46. A method for detecting the presence or absence of *B. pertussis* in a biological sample from an individual, said method comprising:  
performing at least one cycling step, wherein a cycling step comprises an amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said sample with a pair of IS481 primers to produce an IS481 amplification product if a *B. pertussis* IS481 nucleic acid molecule is present in said sample, wherein said dye-binding step comprises contacting said IS481 amplification product with a nucleic acid binding dye; and

detecting the presence or absence of binding of said nucleic acid binding dye to said amplification product,

wherein the presence of binding is indicative of the presence of *B. pertussis* in said sample, and wherein the absence of binding is indicative of the absence of *B. pertussis* in said sample.

47. The method of claim 46, wherein said nucleic acid binding dye is selected from the group consisting of SYBRGreenI<sup>®</sup>, SYBRGold<sup>®</sup>, and ethidium bromide.

48. The method of claim 47, further comprising determining the melting  
5 temperature between said IS481 amplification product and said nucleic acid binding dye,  
wherein said melting temperature confirms said presence or absence of said *B. pertussis*.